In vitro antifungal activity, phytochemical screening and thin layer chromatography profiling of *Impatiens tinctoria* A. Rich root extracts


**Abstract**

**Background:** The emergence of microorganisms’ new resistance mechanism, the emerging and re-emerging of new infectious diseases and side effects of antimicrobials threatening our ability to treat infectious diseases. Therefore, researches for the innovation of new drugs are needed through identification of the best candidate like plants which are used in traditional medicine. In Ethiopia, women dye their palms and nails by a root paste of the study plant (*Impatiens tinctoria* A. Rich) as a beauty treatment, to toughen the skin and to control fungal infections.

**Objectives:** To investigate the antifungal activities, phytochemical screening and Thin Layer Chromatography profiling of aqueous, ethanol and ethyl acetate root extracts of *Impatiens tinctoria* A. Rich.

**Methods:** The roots of *Impatiens tinctoria* A. Rich were collected from Gurage Zone around Butajira town and processed and extracted using solvents of ethyl acetate, ethanol and water. Agar well diffusion and agar dilution methods were used for screening the antifungal activity of the extracts and for determination of minimal inhibitory concentration, respectively. The minimum fungicidal concentration of the extracts was determined and the plant extracts were subjected to phytochemical screening.

**Result:** From the tested fungi *T. rubrum* and *T. mentagrophytes* were more susceptible. The study screened the presence of at least 7 phytochemicals (alkaloids, flavonoids, anthraquinens, terpenoids, glycosides, quinones and saponins) and 6 compounds in the roots of the study plant. Ethyl acetate extract was more potent and broader spectrum of antifungal activities than ethanol and aqueous extracts.

**Conclusion:** The roots of the study plant showed notable antifungal potency. However, further research should be undertaken to elucidate the involved phytochemicals and compounds present in the extracts and mechanism of action which clear out the road for developing new antifungal drugs.

**Keywords:** Antifungal, phytochemicals, thin layer chromatography, root extracts, *Impatiens tinctoria* A. Rich, Ethiopia

**1. Introduction**

Antimicrobial resistance becomes a global concern because new resistance mechanisms are emerging and spreading globally, threatening our ability to treat common infectious diseases [1]. In the other way there is a major challenge to combat new emerged infections since there is no specific treatment against them [2]. In addition to these problems, drugs are sometimes associated with adverse effects on host which include hypersensitivity, depletion of beneficial gut and mucosal microorganisms, immune suppression and allergic reactions [3]. Moreover, in the last few decades drug development is becoming more and more inefficient mainly due to large development costs and low clinical trial drugs success rate (below 12%) [4]. Due to these problems the burden of infectious diseases like fungal infections is growing globally [5]. Though only 15% of the angiosperms (flowering plants) have been chemically investigated for their medical potential, various important drugs were developed from plants and their secondary metabolite constituents [6-8]. The leading effect of traditional healing practices on medicinal plants contribute the discovery of about 25% of modern medicines (like emetine, quinine and berberine), that is, descended from plants primarily used as traditional remedies [8].
Medicinal plants are now more focused than ever due to the isolation of antimicrobial chemicals from them such as alkaloids, terpenoids, flavonoids, tannins and phenolic compounds [9-13]. Therefore, using plants for innovation of new drugs by studying the potential antimicrobial activity of plant derived substances which are used in traditional medicine in different countries is a good alternative solution to treat infectious diseases [9].

The study plant _Impatiens tinctoria _A. Rich belongs to _Impatiens _genera and that of Balsaminaceae family is one of the species of over 1000 species belonging to this family [14].

In Ethiopia women chop or mash the inside of the tubers (roots) of _I. tinctoria _A. Rich in to a paste to dye the palms and nails of the hands and feet a dark reddish color (Figure 2). It is considered a beauty treatment similar to that of henna. It also helps to control fungal infections like ringworm that cause athletes foot (tinea pedis) and to toughen the skin. [15, 19]. Thus, using this information and other ethnobotanical studies [20-22] that support the medicinal value of the plant as a base line the root extracts of the study plant was evaluated for antifungal activities. The preliminary phytochemical screening could be used as a starting point to quantify the identified phytochemical constituents and for further isolation of bio-active compounds. So, the study provides base line information for the development of alternative new drugs.

2. Materials and methods

2.1 Plant material collection

The roots of _I. tinctoria _A. Rich were collected around Butajira town after obtained permission from Butajira town administration. Butajira is located in the Gurage Zone of the Southern Nations, Nationalities and Peoples’ Region, Southern central Ethiopia, 130 km from Addis Ababa. This town has a latitude and longitude of 8°07′N 38°22′E and an elevation of 2131 meters above sea level. The plant material was identified by a botanist, and a voucher specimen deposited in the herbarium of Traditional and Modern Medicine Research Directorate (TMMRD).

2.2 Herbal material preparation

Fresh _I. tinctoria _A. Rich roots were washed with clean water and rinsed with distilled water, cut into pieces and dried at room temperature in shade and lastly milled to powder by using milling machine. Then, the powder was weighed using electronic weighting balance and packed in polyethylene bags to avoid entrance of air and any other contaminant and stored in closed container with proper labeling for further extraction processes.

2.3 Extraction of the collected roots

Different studies on medicinal plants showed as extraction solvents used for extraction has a great influence on the antimicrobial activity of the plant materials [23-25]. Therefore, the powder part of the study plant was extracted using three different solvents. 100 grams of the powdery sample was weighed into each of the three cleaned and dried 2000 ml reagent bottles and 1000 ml of each solvent (ethyl acetate, ethanol and water) was separately added to each of the bottle and shaken (100 revolution per minute) on a shaking orbit machine (VWR DS-500; The Lab World Group, Boston, MA, USA) for 24 hours at room temperature. The mixture was filtered through whatman no.1 filter paper. After filtration the filtrant was repeated for additional two cycles with fresh solvent. The ethyl acetate and ethanol filtrate was concentrated under reduced pressure at 40°C by a rotary evaporator (R-200 Buchi, Switzerland). These concentrated filtrates were transferred to beakers and dried by keeping beakers in water bath by setting at 40 °C to avoid the
remaining organic solvents while water filtrate dried by lyophilizer (freeze dryer). Finally, extracts were kept at 2-8°C [26].

2.4 Antifungal activity of the extracts
2.4.1 Microorganisms
Antifungal activity of I. tinctoria A. Rich root extracts were evaluated on clinical isolated yeast (C. albicans) and on four drug sensitive standard strains of American Type Culture Collection (ATCC) molds that were obtained from microbiology laboratory of TMMRD and National Clinical Bacteriology and Mycology Reference Laboratory of EPHI, Ethiopia. The tested mold strains were T. rubrum (ATCC 28188), T. mentagrophytes (ATCC 18748), A. niger (ATCC 10535) and A. flavus (ATCC 13697).

2.4.2 Inoculum preparation
Inoculums preparation was according to recommended by Clinical and Laboratory Standards Institute (CLSI). All strains were grown in Petridishes containing sabroud dextrose agar medium as refreshment of each strain for actual test by incubating at 25°C for 7 days. Standardization was by taking 3-5 inoculums from a fresh, pure culture of the test organism and making a suspension with sabroud dextrose broth. The absorbance of the prepared suspension was read by uv-visible spectrophotometer (Thermo Scientific Evolution 60s CAT 840210100) with a 1cm light path till obtained an absorbance reading of 0.08 to 0.1 at 625nm which is proportional to 1x106 spores/ml fungi. Then, these suspension diluted with appropriate broth in 1:10 to get 1x105 spore/ml fungi, respectively that used to evaluate the antifungal activity of the extracts parallel to positive and negative controls [27, 28].

2.4.3 Screening antimicrobial activity of the extracts
Agar well diffusion technique was used for screening of antimicrobial activity of the plant extracts. A sterile cotton swab was dipped into the adjusted suspension. The swab rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This removes excess inoculums from the swab. The dried surface of a plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculums. As a final step, the rim of the agar swabbed [27].

The streaked plate punched aseptically with a sterile cork borer that has a diameter of 8mm to form a hole for placing extracts, positive and negative controls. A 100µl volume of the antimicrobial agent or extract solution at desired concentration (100mg/ml, 200mg/ml and 400mg/ml) was introduced into the well. Standard drugs as positive control and diluents as negative control were run simultaneously. Then, the plates incubated at 25°C up to 7 days. The antifungal agent diffuses in the agar medium and inhibits the growth of the fungal strains tested. The presence of inhibition zones were measured by ruler recorded and considered as indication for antifungal activity [29].

2.4.4 Determination of Minimum Inhibitory Concentration
Standard agar dilution method was employed to determine Minimum Inhibitory Concentration (MIC) value of the plant extracts against all screened 5 test organisms. Different concentrations of extracts starting from a highest concentration of 64 mg/ml to 0.0625 mg/ml were prepared by two-fold serial dilution. Then, 2ml of the prepared concentrations were added in to test tubes containing 18 ml of molten agar medium that cooled to 55°C and mixed thoroughly followed by dispensing to the petridish. After dispensing the plates were allowed to solidify at room temperatures. The next procedure was putting inoculums by taking 2µl standardized suspension using micropipette. The inoculums suspension preparation and incubation time was similar to the screening agar well method. The MIC was considered as the lowest concentration which inhibits the growth of the respective fungi under suitable incubation conditions expressed in mg/ml [29].

2.4.5 Determination of Minimum Fungicidal Concentration
Streaks were taken from MIC plates exhibiting invisible growth and sub cultured onto appropriate extract and standard drug free agar plate medium. The plates incubated at 25°C up to 7 days. Then after examined for fungal growth in corresponding to plant extract concentrations in which Minimum Fungicidal Concentration (MFC) was taken as the highest diluted extract that showed absence of any growth [30]. Generally, during the above all antimicrobial assays positive controls, negative controls, sterility controls and growth controls were run parallel to the experimental tests. Discs of amphotericin B (32µg) were used as positive control in well diffusion assays whereas by diluting the powdered form of these standard drugs was used for MIC value determination. Since ethanol and ethyl acetate extracts were dissolved by 5% tween 80 and aqueous extract was dissolved by distilled water, 5% tween 80 for ethanol and ethyl acetate extracts and distilled water for aqueous extract were used as negative control. All assays were performed in triplicate.

2.5 Phytochemical screening
The qualitative phytochemical investigation of each extract of the herbal material was performed to evaluate the presence of different classes of secondary metabolites such as alkaloids, flavonoids, phenols, saponins, tannins and terpenoids. The qualitative results were expressed as positive for the presence and negative for the absence of phytochemicals. It was carried out using standard tests as described below.

Test for alkaloids (Wagner’s Test): Extracts were dissolved individually in dilute HCl and filtered. Then, filtrates of each extract were treated with Wagner’s reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids [10].

Test for flavonoids (H2SO4 test): A fraction of the extracts was treated with concentrated H2SO4 and observed for the formation of orange color [10].

Test for phenols: About 0.5 g of plant extract was added to 1 ml of 10% FeCl3 solution. A deep bluish green coloration was an indication for the presence of phenol [31].

Test for tannins: 0.5 g of plant extract was mixed with 2mL of water and heated on water bath. The mixture was filtered and 1mL of 10% FeCl3 solution was added to the filtrate. A blue-black solution indicates the presence of tannin [32].

Test for free anthraquinones (Borntrager’s Test): About 0.2 g of each extract was shaken with 10 mL of benzene and then filtered. Five ml of the 10% ammonia solution was then added to the filtrate and shaken. Appearance of a pink, red or violet color in the ammonia (lower) phase was taken as the presence of free anthraquinones [33].
2.7 Data Quality Assurance

To ensure the quality of the data all the laboratory works were performed according to the SOPs and internationally accepted principles for testing of medicinal plants by the principal investigator with the help of experienced professionals. Each equipments, supplies, reagents and procedures were adequately controlled. The assessment of the antimicrobial activity, the TLC profiling and phytochemical screening of the extracts was performed at least in triplicate with controls for insuring quality and reproducibility results. There was a continuous monitoring and supervision of the data extraction by advisors. The extracted data was examined for completeness and checked for consistency before entering it into the excel spreadsheet.

2.8 Data analysis and interpretation

Data was entered into excel spreadsheet and analyzed using Minitab 16 software. The statistical differences of the antifungal activity of crude extract on each fungi was carried out by employing one way analysis of variance (Anova) followed by Tukey’s multiple comparison tests. The experimental data was expressed as mean ± Standard Deviation (SD). The result was considered statistically significant at 95% confidence level and P<0.05.

3. Results

3.1 Antifungal activity screening by well method

On this method the study plant root extracts showed different inhibition zone against C. albicans, T. rubrum, T. mentagrophytes, A. niger and A. flavus. The dermatophytes, T. rubrum and T. mentagrophytes, were more susceptible compared to the other fungal species at all tested extract types and concentrations. Ethyl acetate extract showed a higher inhibition of the growth of tested fungi when compared to aqueous and ethanol extracts (P<0.05). The inhibition activity of each extracts was dose dependent. There was no inhibition of C. albicans, A. niger and A. flavus against the aqueous crude extract at 100mg/ml concentration. Among the extracts used aqueous extract has showed significantly lower inhibition for all tested fungi except A. niger (P<0.05). Generally, the antifungal activities of the ethyl acetate extract(at all tested concentrations) in terms of its zone of inhibition were better or in line with the positive amphotericine B (32 µg /ml) against each fungal strain (table 1).

Table 1: Inhibition zone diameter measurement (mm) of crude extracts of *I. tinctoria* A. Rich roots against fungi

<table>
<thead>
<tr>
<th>Extracts Type</th>
<th>Conc. (mg/ml)</th>
<th>Inhibition Zone Diameter (mm), including well diameter (8 mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>C. albicans</strong></td>
</tr>
<tr>
<td>Aqueous</td>
<td>100</td>
<td>8.0±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>7.8±0.6&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>9.7±0.6&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ethanol</td>
<td>100</td>
<td>11.3±0.6&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>12.3±0.6&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>15.7±0.6&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>100</td>
<td>22.7±1.5&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>24.7±1.2&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>27.3±2.1&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
<td>Distilled water</td>
<td></td>
<td>8.0±0.0&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>5% tween 80</td>
<td></td>
<td>8.0±0.0&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Amphotericine B</td>
<td>32 µg</td>
<td>25.3±0.6&lt;sup&gt;ef&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Key notes: Values are expressed as Mean ± SD (n=3). 8.0±0.0<sup>e</sup> = no inhibition (well diameter), “Means” that do not share a superscript letter are significantly different (only column wise) at P<0.05. Conc.-concentration, T. menta. - T. mentagrophytes
3.2 Minimum Inhibitory Concentration and Minimum Fungicidal Concentration of the extracts

Among the tested fungi, *T. rubrum* was the most susceptible with MIC value of 0.7±0.3 mg/ml, 1.0±0.0 mg/ml and 1.0±0.0 mg/ml by ethyl acetate, ethanol and aqueous extracts, respectively, followed by *T. mentagrophytes* which has showed MIC value of 1±0.0 mg/ml, 4±0.0 mg/ml and 8±0.0 mg/ml, respectively. *C. albicans*, *A. niger* and *A. flavus* were more resistant fungal species to the extracts with MIC value of greater than 64 mg/ml for both ethanol and aqueous extracts. However, ethyl acetate extract the MIC value of 16±0.0 mg/ml and 32±0.0 mg/ml were recorded for *C. albicans* and *A. flavus*, respectively (table 2). The MFC in µg/ml was expressed in mg/ml for extracts and in µg/ml for positive control.

### Table 2: MIC and MFC value of tested fungi

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>MIC and MFC</th>
<th>Water extract</th>
<th>Ethanol extract</th>
<th>Ethyl acetate extract</th>
<th>Positive control</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. albicans</em></td>
<td>MIC</td>
<td>&gt;64±0.0</td>
<td>&gt;64±0.0</td>
<td>32±0.0</td>
<td>32±0.0</td>
</tr>
<tr>
<td></td>
<td>MFC</td>
<td>&gt;64±0.0</td>
<td>&gt;64±0.0</td>
<td>64±0.0</td>
<td>64±0.0</td>
</tr>
<tr>
<td><em>T. rubrum</em></td>
<td>MIC</td>
<td>1±0.0</td>
<td>1±0.0</td>
<td>0.7±0.3</td>
<td>2±0.0</td>
</tr>
<tr>
<td></td>
<td>MFC</td>
<td>2±0.0</td>
<td>2±0.0</td>
<td>1±0.0</td>
<td>4±0.0</td>
</tr>
<tr>
<td><em>T. mentagrophytes</em></td>
<td>MIC</td>
<td>8±0.0</td>
<td>8±0.0</td>
<td>2±0.0</td>
<td>4±0.0</td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>MIC</td>
<td>&gt;64±0.0</td>
<td>&gt;64±0.0</td>
<td>16±0.0</td>
<td>6.7±2.3</td>
</tr>
<tr>
<td></td>
<td>MFC</td>
<td>&gt;64±0.0</td>
<td>&gt;64±0.0</td>
<td>16±0.0</td>
<td>8±0.0</td>
</tr>
<tr>
<td><em>A. flavus</em></td>
<td>MIC</td>
<td>&gt;64±0.0</td>
<td>&gt;64±0.0</td>
<td>32±0.0</td>
<td>16±0.0</td>
</tr>
<tr>
<td></td>
<td>MFC</td>
<td>&gt;64±0.0</td>
<td>&gt;64±0.0</td>
<td>32±0.0</td>
<td>32±0.0</td>
</tr>
</tbody>
</table>

**Key notes:** Amphotericin B was the positive control drug. The MIC and MFC values were expressed in mg/ml for extracts and in µg/ml for positive control.

3.3 Phytochemical constituents of the crude extracts

According to the qualitative phytochemical screening study, around 7 secondary phytochemicals were detected at least in one of the three extracts (table 3). The ethyl acetate, ethanol and aqueous extracts of the roots of *I. tinctoria* A.Rich were found to be positive for 6, 5 and 4 tested secondary metabolites, respectively. Glycoicides, saponins and terpenoids were positive for all extracts whereas coumerines, phenols and tannins were negative for all extracts.

### Table 3: Secondary metabolites detected with in aqueous, ethanol and ethyl acetate crude extracts of the root of *I. tinctoria* A.Rich

<table>
<thead>
<tr>
<th>Phytochemical constituent</th>
<th>Water extract</th>
<th>Ethanol extract</th>
<th>Ethyl acetate extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Free anthraquinines</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Coumerines</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Glycoicides</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Quinines</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

**Key notes:** (+) positive, (-) negative

3.4 TLC profiling

TLC analysis of each extract was performed using various combinations of solvent systems till the analysis revealed a better separated constituents. The solvent system containing a mixture of hexane: ethyl acetate (7:5) produced a better separation for ethanol and ethyl acetate extracts. Though various solvent combinations with different proportions tested, the aqueous extract didn’t give well separated constituents of compounds. Of the analyzed mixture dichloromethane: ethyl acetate (7:2) produced a little bit better separated components. The migrated constituents, formed by the solvent system, observed under UV-light at a wavelength of 254nm, revealed the presence of 6, 4 and 1 compounds with in ethanol, ethyl acetate and aqueous extracts, respectively (Figure 3).

![Fig 3: TLC fingerprint of A Ethyl acetate extract and B Ethanol extract](image)
these most prevalent Trichophytons (T. rubrum and T. mentagrophytes) [38] than the other tested fungi. Of the screened 10 phytochemicals seven were positive in which some were positive for the three extracts or two extracts and others for one extract of I. tinctoria A.Rich roots. Therefore, the phytochemical composition of the three extracts differ by type and those similar type phytochemicals that found in different extracts might also differ by concentration. Different studies support the extraction solvents used for extraction has a great influence on the type and concentration of phytochemicals screened [39, 40]. The solvents used in the extraction of the plant materials might not only contribute for the difference in kind of these secondary metabolites among the three extracts but might also contribute for the difference in concentration. The study revealed the presence of terpenoids, saponins and glycoicidies with in the three extracts of I. tinctoria A. Rich roots. Previously, numerous phytochemicals such as alkaloids, anthraquinins, flavonoids, terpenoids, glycosides, quinones and saponins have also revealed from many medicinal plants that had health benefits such as antimicrobial effect through different mechanisms [12, 32, 41]. For instance, a study on the isolate of terpenoids from the medicinal plant Trichodesma amplexicaule Roth found the presence of antifungal activity of this phytochemical [42]. Therefore, the isolated phytochemicals from the root extracts of the study plant might help for the potency of antifungal activity. Alkaloid was the other phytochemical screened from ethyl acetate and ethanol extracts but not in aqueous extract. The presence of this phytochemical in the ethyl acetate and ethanol extract could contribute for their better antimicrobial activities than the aqueous extract. This idea supported by a study in Slovakia on antibacterial and antifungal effect of Mahonia aquifolium crude extract and its major isolated alkaloids berberine and jatrorrhizine on 20 bacterial and fungal tested strains revealed that the isolated alkaloids berbeine showed more susceptibility on most the fungal tested strains. In addition to the above phytochemicals only ethyl acetate extract was positive for flavoinodes that might have a great contribution for its antifungal activity as different studies support the antimicrobial effect of this phytochemical [33, 44].

The study detected greater number of phytochemicals in ethyl acetate extract than ethanol and aqueous extracts. Of the three extracts the ethyl acetate extract has stronger and broader spectrum of antimicrobial activities as observed from mean inhibition diameter measurements and the consistent MIC and MFC values. This might be due to the synergistic effect of the secondary metabolites as many studies assure synergistic effect of secondary metabolites increase the antimicrobial potency of plant extracts [45-47]. Additionally, the bioactive phytochemicals that only found in ethyl acetate extract might have relatively more antimicrobial activity than others. On the other way not only by type but also the concentration of the phytochemicals present in the ethyl acetate extract might be greater than ethanol and aqueous extracts that could increase the antimicrobial activity of ethyl acetate extract than ethanol and aqueous extracts.

5. Conclusion

According to this study, the plant extracts showed varying antifungal activities in which T. rubrum and T. mentagrophytes were more susceptible at all corresponding extract types and concentrations compared to other tested fungi. Therefore, this antifungal profile of the study plant on this study support applying of I.tinctoria A.Rich roots paste topically for control of certain fungal infections though further studies are needed. The antifungal activity of the study plant showed notable extraction solvent dependent manner in which ethyl acetate extract showed more antifungal activity in most of the tested fungi compared to the aqueous and ethanol extracts. This might be due to the type and concentration of phytochemicals present in each extract.

Ethics approval and consent to participate
Not applicable
Consent for publication
Not applicable

Competing interests
No competing interests

Funding
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Availability of data and materials
All raw data were generated at Ethiopian Public Health Institute. The corresponding author has all fabricated data that support the findings of this study and majority of them are presented in the main manuscript.

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Author Contributions

Designed the experiments: Silesi Degu, Adane Bitew, Negero Gemeda, Abiy Abebe
Collection of plant material: Silesi Degu
Extraction, phytochemical analysis and TLC profiling: Silesi Degu, Abiy Abebe, Samson Taye, Bihonegni Sisay, Asfaw Berihun, Eyob Debebe, Aliyi Amano, Netafe Fekadu, Tzion Kasahun
Performed the bioassay experiments: Silesi Degu, Abiy Abebe, Samson Taye, Hirut Gemeda, Reki Ashiber
Analyzed the data: Silesi Degu, Negero Gemeda, Abiy Abebe
Wrote the paper: Silesi Degu
Over all advising and technical support: Adane Bitew, Negero Gemeda, Abiy Abebe

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